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Generation of a monoclonal antibody to mouse C5 application in an ELISA assay for detection of anti-C5 antibodies.

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We have generated a monoclonal antibody with specificity for the fifth component of mouse complement (C5). This antibody precipitates the two chains of C5 from normal mouse serum and inhibits C5-dependent hemolysis in a functional complement test. In this study we describe its application in an enzyme-linked immunoadsorbent assay (ELISA assay) for the detection of anti-C5 antibodies in serum. Monoclonal anti-C5 coupled to wells of an ELISA plate specifically binds C5 from unfractionated normal mouse serum. This subsequently serves as antigen to bind anti-C5 serum antibodies. By this approach we have circumvented the need for extensive purification of C5 from serum which would be required if C5 was directly coupled to ELISA plates as antigen. Serum antibodies from C5-immunized mice bound with high avidity to wells containing normal serum as antigen source in amounts representing 1 microgram to 250 ng C5. There was no antibody binding to wells containing C5-deficient serum as antigen source. The immune reaction was detected by development with enzyme-coupled goat-anti mouse Ig antibodies specific for various mouse Ig subclasses. This method allows the qualitative characterization of immune responses to mouse C5 which is an ideal model for a natural self antigen in studies of immunological tolerance.

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